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RESEARCH ARTICLE

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From the juvenile to the adult vegetative phase in olive seedlings: the transition along the stem axis

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Abstract

Sexual reproduction in olive is carried out for purposes such as breeding. The seedlings evolve from the juvenile to the adult stage, and until now, only the discrete developmental phases have been investigated in detail. However, the transition process has been poorly studied in fruit trees, especially in olive. In this paper, juvenile to adult transition has been explored in 30 olive seedlings coming from the Table Olive Breeding Program of the University of Sevilla, Spain. Despite of the great variability found in the olive leaf morphological parameters, mean values increased linearly from the bottom (juvenile) to the top (adult tissue) of the seedling. An upward lineal decrease in the rooting ability was also observed for the set of seedlings evaluated. No significant differences were found for the maximum net photosynthesis (P_{Nmax}) or maximum stomatal conductance (g_{smax}), although the lowest values were measured at <0.5 m. For all of the analysed parameters, the transitional tissue showed intermediate values. These results show for the first time in olive that the transition along the seedling stem axis follows a clear lineal tendency with a stepwise loss of juvenile characters being the shift from juvenile to adult phase in olive not an abrupt change but a gradual process. The usefulness of a fibre optic probe with a reduced sampling surface coupled to near-infrared spectroscopy (NIRS) was evaluated. NIR analysis has been confirmed to be a useful tool to discriminate the juvenile and adult leaves, but not the transition ones.

Additional key words: *Olea europaea* L.; olive breeding; leaf morphology; rooting ability; NIRS.

Introduction

Higher plants progress through different growing phases during their post-embryonic development. The shoot undergoes an aging process recorded as the variation in characters or structures along the axis from the juvenile to the adult stage (Poethig, 1990; 2003; 2010). The juvenile period is the time during which a plant coming from seeds cannot be induced to flower (Meilan, 1997; Jones, 1999) and it is therefore unproductive under natural growing conditions. The onset of the reproductive phase is associated with changes in vegetative morphological characteristics of the plant.

Sexual reproduction is essential to produce variability in tree breeding programs by crossing. However, the juvenile period of the seedlings produced can reach up to 20 years, as in a variety of tree crops (Fontanazza & Baldoni, 1990; Poethig, 2002; Brunner & Nilsson, 2004).

The olive (*Olea europaea* L.) is a polymorphic tree, so that juvenile leaves clearly differ from those coming from adult tissue. Thus, the evaluation of the leaf morphological parameters has allowed for discrimination between the juvenile and adult vegetative phase (Rapoport, 2008; Moreno-Alías *et al.*, 2009).

The juvenile-to-adult phase change can be described by a characteristic shape called the juvenility cone

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Abbreviations used: DT (detrending); g_{smax} (maximum stomatal conductance); GH (Mahalanobis distance modified); H (Mahalanobis distance); IFAPA (Instituto de Investigación y Formación Agraria y Pesquera); NIRS (near-infrared spectroscopy); PCA (principal component analysis); PLS2-DA (partial least squares discriminant analysis); P_N (net photosynthesis); P_{Nmax} (maximum net photosynthesis); R^2 (coefficient of determination in the calibration); r^2 (coefficient of determination for the cross validation); SEC (standard error of calibration); SECV (standard error of cross validation); SNV (standard normal variate).

(Hartmann *et al*., 2002), which has been studied in olive trees by Moreno-Alías *et al*. (2010) and Suárez *et al*. (2011). The factors affecting the length of the juvenile period in olive trees have also been studied, such as the parent genotype (Santos-Antunes *et al*., 2005; De la Rosa *et al*., 2006; Suárez *et al*., 2011; Moral *et al*., 2013) and the early vigour of the seedlings (De la Rosa *et al*., 2006; Rallo *et al*., 2008; Hammami *et al*., 2011).

In other species, different phases of tree development present different rooting abilities, with juvenile forms showing greater rooting aptitude (Tao *et al*., 1994; Hartmann *et al*., 2002; Bhusal *et al*., 2003; Dolcet-Sanjuan *et al*., 2004; Kibbler *et al*., 2004).

The change from juvenile-to-adult phase usually implies not only morphological but also anatomical and biochemical changes. In olive, it is known that the mesophyll thickness, the layers of palisade parenchyma and the quantity of the peltate trichomes differ between the two phases (Moreno-Alías *et al*., 2009). The peltate trichomes reduce the diffusion of water vapour (Proietti & Palliotti, 1997) and protect the photosynthetic activity by preventing UVB-induced stomatal closure (Grammatikopoulos *et al*., 1994). Therefore, all of these changes may have functional significance for photosynthesis and stomatal conductance as in other species (James & Bell, 2000; Kubien *et al*., 2007; Jaya *et al*., 2010).

Studying the juvenile-to-adult vegetative phase transition through morphological, biochemical or physiological markers is a long and tedious process. Near-infrared spectroscopy (NIRS) is an inexpensive and easy-to-use analytical procedure. It requires small quantities of samples and involves little or no preparation. NIR spectra have been used in olive either to predict olive oil fruit traits (León *et al*., 2004; Armenta *et al*., 2010), table olive fruit traits (Morales-Sillero *et al*., 2011), nutritive composition of olive leaves (Fernández-Cabanás *et al*., 2008) or to discriminate between juvenile and adult leaves (León & Downey, 2006). However, no references have been found in the scientific literature on the use of portable instruments or fibre optic probes with reduced sampling surface for olive leaf NIR analysis, which could save significant time during the determinations and even allow for field determinations.

In summary, the change from the juvenile to the adult stage in trees is a complex process in the stem axis. Although juvenility in olive species has been previously studied, there is still limited information about this phenomenon, and particularly on the transition along the shoot from juvenile to adult stage. Up to date, most of the papers have been focused on the description of discrete and stable developmental phases: juvenile or adult, which is an oversimplification (Poethig, 1990).

The aims of this work were first to study the juvenile-to-adult vegetative phase transition along the stem axis in olive seedlings measured as variations in morphological leaf parameters, rooting ability of cuttings and photosynthesis and stomatal conductance and, second, to study the usefulness of a fibre optic probe with a reduced sampling surface coupled to a NIR spectrophotometer to measure the transition along the axis of olive seedlings.

Material and methods

Plant material

The study was performed on 30 unpruned 5-yr old olive tree seedlings from the University of Sevilla Table Olive Breeding Program. In order to be sure that these seedlings had overcome juvenility, they were selected from a previous research (Suárez *et al*., 2011) were first flower to ground distance in olive seedlings was recorded, and thus, the chosen trees had already flowered below 1.5 m height at the time of data collection. Seedlings were obtained either by controlled crosses between olive cultivars or by open pollination performed in 2004: 'Manzanilla de Sevilla' × 'Changlot Real' (12), 'Changlot Real' × 'Manzanilla de Sevilla' (11), 'Manzanilla de Sevilla' Open pollination (6), 'Manzanilla de Sevilla' × 'Gordal Sevillana' (1). Seeds were germinated and subsequent seedlings grown under forcing conditions as described by Santos-Antunes *et al*. (2005). Trees were planted in 2006 at the experimental farm of the Instituto de Investigación y Formación Agraria y Pesquera (IFAPA) "Las Torres" in Alcalá del Río, Sevilla, Spain (37°30´ N, 05°57´ W, 11.0 m asl). They were planted in ridges with a layout of 5 by 3 m. Pest control, soil fertilisation, and soil management were conducted in accordance with the regional commercial uses.

Trial 1. Juvenile-to-adult vegetative phase transition along the olive stem axis: the leaf morphological parameters

For each olive tree, leaf samples (six replications of fully developed leaves from the middle region of the

annual shoots) were taken in spring at three sampling heights above the ground: 0.5 (or on the lowest branch of the tree at 0.6-0.8 m), 1.0, and 1.5 m. The total area (mm^2), length (mm) and width (mm) were measured with the Delta-T SCAN image analysis software.

Trial 2. Juvenile-to-adult vegetative phase transition along the olive stem axis: the rooting ability of the cuttings

The rooting ability of each olive tree was evaluated by mist propagation of semi-hardwood olive cuttings according to the procedure described by Caballero & Del Río (2008). Four to twelve cuttings (depending on suitable plant material) of 160-180 mm in length, removing all leaves but the four on the two top nodes, were taken in the spring for each sampling height above ground (0.5, 1.0, and 1.5 m along the stem axis), resulting in a total of 1006 cuttings.

The base of the cuttings was dipped into 3-Indolbutyric Acid (3500 mg kg^{-1} auxin alcoholic solution) for 10 sec and then left outside for 10 min to dry. The cuttings were randomly placed on a mist bench inside a greenhouse with perlite as the rooting medium and bottom heat (22°C). Micro-sprinklers were placed at a 1 m distance, and mist cycles of 25 seconds every 5 min were carried out from 05.00 to 19.00 h GTM each day. The cuttings were treated with fungicide (Benomilo, 0.5 g L^{-1}) against *Cycloconium oleaginum* Cast.

Two months later, the cuttings were taken out of the mist bench and the following measurements were performed: number of live cuttings, presence of necrosis, presence of callus, presence of roots, number of roots per cutting, total root length (mm), and fresh weight of the roots (g). The following traits were then calculated: percentage of rooted cuttings, mean number and length of roots per cutting (mm), root fresh weight per cutting (g), percentage of cuttings with callus and the percentage of surviving cuttings.

Trial 3. Juvenile-to-adult vegetative phase transition along the olive stem axis: net photosynthesis and stomatal conductance

The maximum net photosynthesis (P_{Nmax}) and maximum stomatal conductance (g_{smax}) were measured in eight olive trees in June. Six fully expanded leaves of the current year, in sun-oriented parts of the canopy, were sampled per tree at three sampling heights above ground: <0.5, 0.5-1.0 and >1.5 m. The measurements were made between 08.00 and 10.00 h GTM, when the stomatal conductance was at its maximum, with two LI-6400 portable photosynthesis systems (Licor Inc., USA).

Trial 4. Discrimination of the juvenile and adult leaves by fibre optic NIR spectroscopy

Leaf samples from Trial 1 were scanned on a Foss-NIRSystems 6500 SY-II monochromator (Foss NIRSystems, Silver Spring, MD, USA) interfaced to a fibre optic probe module (NR-6775), from 800 to 2200 nm, every 2 nm (spectral bandpass 10 nm ± 1 nm). The interactance-reflectance NIR spectra of the adaxial surface (León & Downey, 2006) of 2-3 intact leaves samples placed over an aluminium foil support were collected.

All the spectra were manipulated and processed, and all the calibration equations were obtained using the WINISI software, vers. 1.5 (Infrasoft International, State College, PA, USA).

For structuring the calibration set, an initial principal component analysis (PCA) was performed to calculate the centre of the population and the distance of the samples (spectra) from that centre in an n-dimensional space, using the Mahalanobis distance modified (GH) and removing samples with a statistical value greater than three. Mahalanobis distance (H) takes into account the correlation in the data, since it is calculated using the inverse of the variance-covariance matrix of the data set of interest. The GH value is a modification of the Mahalanobis distance, H, in which H*H (H squared) is divided by the number of dimensions, p, used to derive H. GH values give the distance of one sample to the population centre.

Discriminant models were constructed to classify the adult, transition, and juvenile leaves using partial least squares discriminant analysis (PLS2-DA) for supervised classification (Naes *et al*., 2002), using the "discriminant equations" option in the WINISI vers. 1.50 software package (ISI, 2000). Standard normal variate (SNV) and detrending (DT) mathematical pretreatments were used to correct for scatter phenomena (Barnes *et al*., 1989). Furthermore, nine derivative mathematical treatments were tested: 1,5,5,1; 1,10,5,1; 1,10,10,1; 2,5,5,1; 2,10,5,1; 2,10,10,1; 3,5,5,1;

3,10,5,1; and 3,10,10 where the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the smoothing segment, and the fourth is the second smoothing segment (Shenk *et al*., 1989).

The precision of the models obtained was evaluated using the percentage of correctly classified samples and the statistical values obtained for the calibration. The main calibration statistics are as follows: the standard error of calibration (SEC), the coefficient of determination in the calibration (R^2), the standard error of cross validation (SECV), and the coefficient of determination for the cross validation (r^2). The best calibrations were selected based on the higher r^2 and lower SECV and classification error values.

Statistical analysis

Fit to linear and quadratic regression with seedling height were performed to confirm the differences in leaf morphology, photosynthesis and stomatal conductance, and rooting ability of cuttings along the stem axis due to the juvenile-to-adult vegetative phase transition. 'Statgraphics Plus' software (vers. 5.1, Manugistics Inc., USA) was used for the statistical analyses. To achieve normality and homoscedasticity of the data, Box-Cox power transformations (Box & Cox, 1964) were performed.

Results

Trial 1. Juvenile-to-adult vegetative phase transition along the olive stem axis: leaf morphological parameters

To study the juvenile-to-adult vegetative phase transition along the each olive seedling axis, the following parameters (range: maximum and minimum and mean value) of leaves sampled at three heights above the ground (0.5, 1.0 and 1.5 m) were evaluated in 30 olive seedlings: length (mm), width (mm), and area (mm^2); the shape index was calculated as length/width (L/W) (Fig. 1). All of the analysed leaf morphological parameters (width, length, area, and the shape index of the leaves) showed a wide variability, ranging from 20.1% (width) to 51.5% (area) (Fig. 1). When sorted by sampling height (0.5, 1.0 and 1.5 m) all of these parameters increased linearly ($p < 0.001$) from the bottom (0.5 m), where the smallest values were found, to the top (1.5 m), where the greatest values were measured. The intermediate mean values were recorded at an intermediate sampling height (1.0 m). The range of variation of these parameters did not overlap among the three sampling heights (Fig. 1).

Trial 2. Juvenile-to-adult vegetative phase transition along the olive stem axis: rooting ability of cuttings

To evaluate the juvenile-to-adult transition, the rooting ability of cuttings sampled at three heights above ground (0.5, 1.0 and 1.5 m) was assessed. Significant differences were found in all of the studied parameters in relation to the rooting ability of the cuttings (Table 1). In fact, the percentage of rooted cuttings ($p < 0.001$), the number ($p < 0.001$) and length ($p < 0.01$) of roots, the percentage of cuttings with callus ($p < 0.01$), and the percentage of surviving cuttings ($p < 0.05$) were higher at the bottom (<0.5 m), and they decreased linearly toward the top (Table 1). These results were significant despite the variability found for the analyzed parameters, for example, the rooting percentage of cuttings ranged from 8.3% (genotype which has less rooted) to 77.8% (genotype most successful rooting) and the percentage of cuttings with callus ranged from 5.6% to 86.1%.

Trial 3. Juvenile-to-adult vegetative phase transition along the olive stem axis: net photosynthesis and stomatal conductance

The maximum net photosynthesis (P_{Nmax}) and maximum stomatal conductance (g_{smax}) were measured along the olive stem axis to assess the juvenile-to-adult vegetative transition. The g_{smax} increased with sampling height (0.14, 0.15 and 0.16 mol m^{-2} s^{-1} from bottom to top), although the differences were not statistically significant. No significant differences were found in the P_{Nmax} values (10.73, 12.03 and 11.28 mmol m^{-2} s^{-1}, from bottom to top), although the smallest value was obtained at <0.5 m. A high variability has also been found in this trial. The coefficient of variation for both g_{smax} and P_{Nmax} ranged between 55.3 and 84.6%.

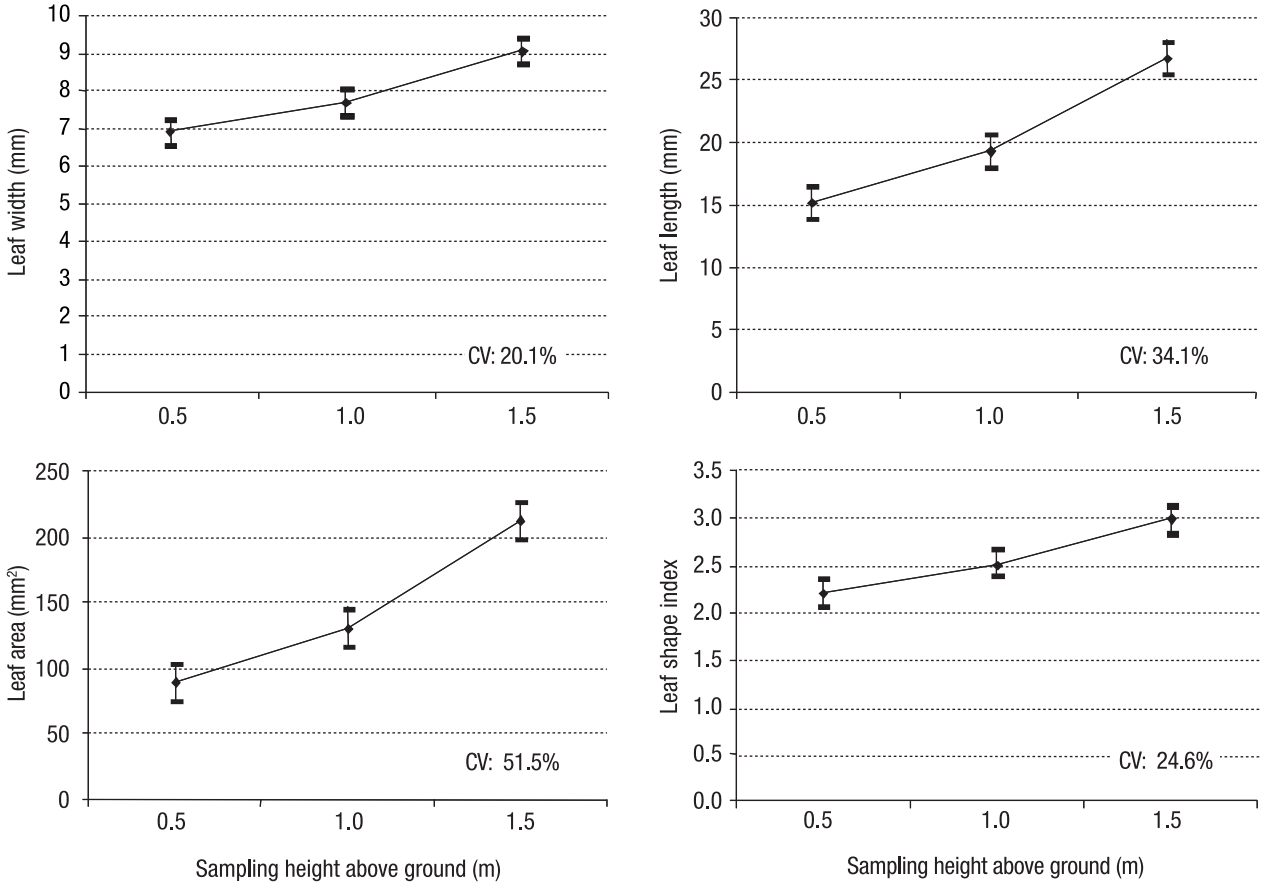

Figure 1. Range (maximum and minimum) and mean values of the morphological leaf parameters along the olive seedling stems. Fit to linear tendency ($p < 0.001$) with sampling height (m) above ground. CV: coefficient of variation.

Table 1. Rooting ability of the cuttings along the olive seedling stems. Percentage of rooted cuttings, number of roots per cutting, length of roots, root fresh weight per cutting, percentage of cuttings with callus and percentage of survival cuttings

	Sampling height (m) above ground			Tendency[1]
	0.5	1.0	1.5	
Rooting of cuttings (%)	50.8	43.8	24.0	L ($p < 0.001$)
Root number per cutting	13.6	10.6	7.9	L ($p < 0.001$)
Root length (mm)	29.8	31.3	18.7	L ($p < 0.01$)
Root fresh weight per cutting (mg)	287	397	262	Q ($p < 0.05$); L (ns)
Cuttings with callus (%)	54.6	47.1	39.1	L ($p < 0.01$)
Survival (%)	84.9	88.3	78.3	L, Q ($p < 0.05$)

[1] L, Q: fit to linear or quadratic tendency, respectively; ns: not significant.

Trial 4. Discrimination of juvenile and adult leaves by fibre optic NIR spectroscopy

Discriminant models developed for leaf samples spectra located at the three studied heights above the ground were not able to clearly discriminate the transition group, thus specific models were computed for the classification of adult and juvenile samples.

Juvenile and adult samples (0.5 and 1.5 m heights, respectively) scanned with the fibre optic probe sho-

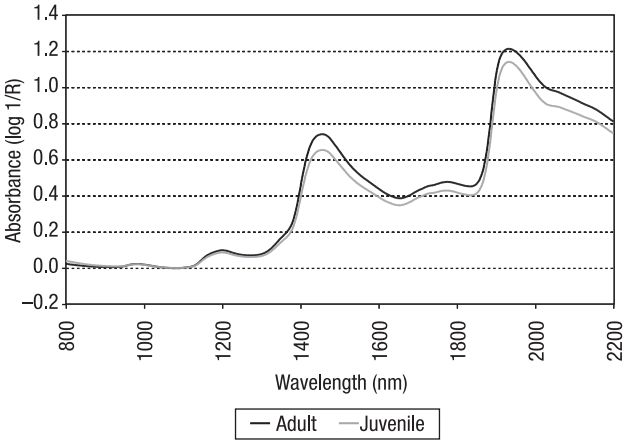

Figure 2. Average spectra corresponding to juvenile and adult leaf samples (<0.5 and >1.5 m heights, respectively) scanned with the NIR fibre optic probe.

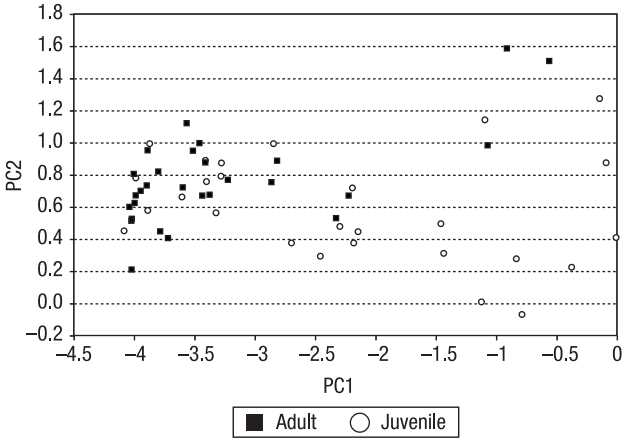

Figure 3. Principal component score plot for the juvenile and adult leaf samples (<0.5 and >1.5 m heights, respectively) scanned with the NIR fibre optic probe.

wed average spectral patterns with noticeable water absorption bands located at 1454 and 1932 nm and CH absorption bands (cellulose and oil) at 1200 and 1726 nm (Fig. 2). In this figure, it is also remarkable that the absorbance values were higher for adult leaves, especially around the water absorption bands.

However, when the individual spectra were compared by performing a PCA with SNV and DT and derivative 1,5,5,1 as spectral pre-treatments, a clear discrimination pattern was not found, even for the juvenile and adult samples (Fig. 3). Although the two first principal components accounted for over 95% of the spectral variance, they were not able to clearly differentiate all of the juvenile and adult leaves.

The percentage of correctly classified samples ranged between 63.64 and 81.82%, with the lower value linked to the lack of spectral pre-treatment (Table 2).

Discussion

The development of the olive seedlings from the juvenile to the adult stage is associated with changes in vegetative morphological characteristics of the plant, and until now, only the discrete developmental phases have been investigated in detail. Significant differences in several leaf morphological and histological parameters between adult and juvenile olive leaves were reported in previous works; *e.g.,* the juvenile leaves of olive trees are shorter and thicker and the branches present shorter internodes (Rapoport, 2008). Moreno-Alías *et al*. (2009) differentiated the two phases based on the size and weight parameters and the leaf anatomy of olive plants grown from seeds, including the leaf size, form, mesophyll thickness, layers of palisade parenchyma, and the quantity of peltate trichomes. These works compare juvenile *vs* adult tissue and they have not always been performed on the same seedling. However the tissue of the transitional zone has not been studied in the stem axis seedling.

In this study, juvenile to adult transition was explored in 30 genetically different olive seedlings and the comparisons have been performed at seedling level through an integrated approach that included the evaluation of very different traits (leaf morphology, rooting ability, net photosynthesis and stomatal conductance) at three sampling heights above the ground (0.5, 1.0 and 1.5 m) at the axis of each seedling. The results show for the first time in olive a significant lineal tendency along the stem axis, from juvenile to adult tissue, in the measured morphological leaf traits and in the rooting ability. Although these lineal responses were highly significant it should be taken into account that the models are based on measurements in only three positions along the axis.

Leaf morphological parameters measured in the Trial 1 (Fig. 1) increased linearly from the bottom to the top showing a transition along the axis and a stepwise loss of juvenile characters, and therefore, the juvenility with increasing distance of the leaves from the trunk base. Other researches carried out in olive and other species (Poethig, 1990; Moreno-Alías *et al.,* 2009) found that juvenile leaves are smaller than adults; this study confirms these findings and adds that the transition tissue shows intermediate values for length, width and area of the leaves according to a linear trend.

Table 2. The percentage of total leaves correctly classified (% CC) and those correctly classified as juvenile and adult leaves by the NIR PLS2-DA models

Math. treatment[1]	Derivative	No. of factors	SECV[2]	% CC		
				Total	Juvenile	Adult
None	None	2	0.48	63.64	51.85	75.00
None	1,5,5,1	4	0.44	80.00	77.78	82.14
None	1,10,5,1	4	0.44	80.00	77.78	82.14
None	1,10,10,1	4	0.44	80.00	77.78	82.14
None	2,5,5,1	3	0.43	76.36	70.37	82.14
None	2,10,5,1	4	0.45	76.36	74.07	78.57
None	2,10,10,1	5	0.45	76.36	77.78	75.00
None	3,5,5,1	2	0.45	74.55	74.07	75.00
None	3,10,5,1	3	0.43	74.55	66.67	82.14
None	3,10,10,1	3	0.43	74.55	66.67	82.14
SNV+DT	None	4	0.46	72.73	66.67	78.57
SNV+DT	1,5,5,1	3	0.45	74.55	59.26	89.29
SNV+DT	1,10,5,1	3	0.45	76.36	62.96	89.29
SNV+DT	1,10,10,1	3	0.45	76.36	62.96	89.29
SNV+DT	2,5,5,1	2	0.43	72.73	66.67	78.57
SNV+DT	2,10,5,1	4	0.45	76.36	74.07	78.57
SNV+DT	2,10,10,1	4	0.46	76.36	74.07	78.57
SNV+DT	3,5,5,1	2	0.42	76.36	74.07	78.57
SNV+DT	3,10,5,1	2	0.42	78.18	74.07	82.14
SNV+DT	3,10,10,1	3	0.41	81.82	70.37	92.86

[1] SNV: standard normal variate; DT: detrending. [2] SECV: standard error of cross validation.

Also the significant differences obtained in rooting ability of cuttings in the Trial 2 measured as the percentage of rooted cuttings, the number and length of roots, the percentage of cuttings with callus, and the percentage of surviving cuttings (Table 1), confirm the upstream transition from juvenile-to-adult along the seedling axis, characterised by a lineal decrease of rooting ability in adulthood. It is well-known that cuttings from juvenile apple, pear, cherry, and other species seedlings root better than those from older plants (Hartmann *et al*., 2002; Bhusal *et al*., 2003; Kibbler *et al*., 2004), and this has been assumed to happen in olive. However, the lineal transition of rooting ability along the seedling stem axis has not been previously described in the olive species, until now (Table 1). Rooting ability is, in fact, one of the practical applications of a better understanding of the vegetative phase transition. Our results also show significant differences between different trees in the rooting percentages, which confirmed the effect of variety on the rooting ability (Suárez *et al*., 1990).

The transition from the juvenile-to-adult vegetative phase involves anatomical changes (Rapoport, 2008; Moreno-Alías *et al*., 2009) and changes in the biochemical composition of the leaves (Fernández-Lorenzo *et al*., 1999) and some authors also note differences in stomatal conductance and net photosynthesis (Sismilich *et al*., 2003; Kubien *et al*., 2007). In Trial 3, there is an upstream increase of stomatal conductance although statistically not significant, and no clear pattern for net photosynthesis (highest value in transition zone and lowest at the bottom) has been found. These results could be explained by the high coefficient of variation for both g_{smax} and P_{Nmax}, which indicates a high variability between seedlings.

The mean values of the carbon dioxide assimilation rate and net photosynthesis (P_N) obtained in this study were low, as it is usual in olive leaves. Maximum values of 22 mmol m^{-2} s^{-1} have been previously reported (Proietti & Palliotti, 1997; Proietti *et al*., 1999; Moriana *et al.,* 2002). Compared with other fruit species, the low photosynthetic capacity of olive leaves seems to be related to leaf morphological characteristics, such as the thickness of the cell walls, the presence of trichomes, and the low density of the photosynthetic reaction centres (Bongi & Palliotti, 1994).

Spectral patterns associated to average NIR spectra of juvenile and adult leaves were consistent with the preliminary results described by León & Downey

(2006) about the position of main absorption bands and higher absorbance values for adult average spectrum. It could be concluded that average spectral values of both group of studied leaves have different features, supporting the results obtained in Trials 1 to 3. Nevertheless, the use of individual spectra in PCA showed some discrepancies. The presence in Fig. 3 of a group of juvenile leaf samples easily identified at the bottom right corner and a second group including juvenile and adult leaf samples could be explained primarily by the presence of samples that were wrongly assigned to their class group. The trees used in this study were small in size, with few leaves on each branch, which may have included juvenile and adult leaves, and the number of scanned samples was reduced (2 or 3). This could lead to the inclusion of samples with different developing stages in the same group. Nevertheless, we should reject this explanation, as Trial 1 performed in this work on the same group of leaf samples provided a clear discrimination of juvenile and adult leaves.

Discriminant models developed by PLS2-DA (Table 2) showed adequate percentage of total correctly classified samples. The classification errors for juvenile leaves were higher than for the adult leaves. One possible explanation is that branches located below 1 m height may include both juvenile and transition leaves, while branches above 1.5 m may be more uniform in their composition.

The results of this work confirm that the morphological parameters of leaves and the rooting ability of shoots can be used as markers to identify the adult vegetative phase in olive trees, in addition to the presence of the first flowers. Moreover, the use of these parameters allows for the analysis of how the juvenile-adult transition takes place along the seedling stem axis. The values of the analysed morphological leaf parameters increased linearly upstream from the base (where juvenile tissue is located) to the top (adult tissue) of the trees, while the rooting ability of cuttings taken from the same olive seedlings decreased, also linearly, along the stem axis. For all of the analysed parameters, except maximum net photosynthesis, the transitional tissue showed intermediate values, which points out that the shift from juvenile to adult stage takes place gradually along the stem.

The use of fibre optic and portable instruments may be helpful for the identification of adult and juvenile phases in the field, especially in breeding programs, avoiding the tedious analysis required for these determinations. Although juvenile (0.5 m high) and adult (1.5 m high) leaves were clearly discriminated, the feasibility of NIR spectroscopy for the identification of leaf samples in the transition phase (1.0 m high) was not accurate. Since the transition phase from juvenile to adult is a rather complex and gradual process, and not a discrete stage, it may not be detected by NIRS analysis of leaves in a specific moment.

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